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EDITORIAL

Mutational analysis of patients with cystinuria detected by a genetic screening network: Powerful tools in understanding the several forms of the disorder

Cystinuria, an inherited error of membrane transport across renal and intestinal epithelium, is an important cause of renal stone disease with a frequency between 1/2500 live births in Israelis of Libyan origin to 1/15,000 in North Americans [1]. Of all natural amino acids, cystine (the mixed disulfide form) is the least soluble in aqueous solutions, particularly at normal urine pH values between 5.0 and 7.0 [2]. Indeed, it is this relative insolubility of cystine that results in morbidity, since recurrent renal stones and infection are the main features of the disorder. It has been recognized since 1966 that there are three distinct variants of this disorder [1]. Parents of type I patients excrete normal amounts of cystine (0 to 100 mmol/g creatinine), those with type III excrete intermediate amounts (100 to 600 mmol/g creatinine) and type II excrete greater amounts of cystine (990 to 1740 mmol/g creatinine). Moreover genetic compounds of cystinuria, such as type I/III can occur. Historically, it has been difficult to define the clinical features of each type, particularly in terms of propensity to stone formation, since most patients come to medical attention only after a symptomatic stone event and anticipatory genetic markers have not been readily available. Two publications in the current issue of *Kidney International* present information that helps to better define the clinical features and probability of stone formation in the first decade of life of the various forms of disease, using both a population and genomic approach [3, 4].

Since the discovery in 1993 of rBAT [5], the gene encoding the transporter for cystine and dibasic amino acids (ornithine, arginine and lysine) transport, it has been possible to seek out mutations or deletions of SCL3A1, as the gene is now termed, in the human gene bank. Evident in subjects with cystinuria [3, 4, 6], this gene is located on chromosome 2 and clearly represents the site of mutation for patients with type I or "classical" cystinuria [7]. Type I patients can be relatively easily identified because their parents excrete normal amounts of cystine, and because they commonly have two mutations in the SCL3A1 gene sequence.

Patients with types II or III do not have mutations in SCL3A1. Indeed, it is probable that the gene responsible for types II and III cystinuria is found on chromosome 19q13.1 [8]. This is the form of cystinuria found in the Israelis of Libyan origin [8]. This gene has not been cloned, but it presumably codes for a cystine transporter at a different nephron site than the proximal straight S3 segment where the SCL3A1 gene product (transporter protein) is found by *in situ* hybridization analysis [9].

In this issue, Rozen and Goodyer and their collaborators have examined 23 children identified by the Quebec screening program, and have characterized these children in terms of (*1*) disease type; (*2*) history of stone formation; and (*3*) presence of an SCL3A1 mutation. The value of the Quebec population is that they have been observed on a longitudinal basis, since detection of disease occurred soon after birth. Moreover, the parental excretion of urinary cystine has also been assessed. Patients with type I/I cystinuria, in which parents excrete cystine within the normal range, had at least two mutations of the SCL3A1 gene in nearly every instance. Between the ages of 1 and 7, the urinary cystine excretion rate exceeded the solubility threshold at a given pH on 70 percent of visits. Fifty percent of these type I/I patients formed stones in the first decade of life.

Those 12 patients who were compound I/III subjects, wherein one parent excretes normal urine cystine and the other excretes moderately elevated amounts, excreted less cystine than type I/I patients and formed no stones in the first decade. In only 22% of visits did urine cystine concentration exceed the theoretical solubility threshold. Moreover only one SCL3A1 mutation was found in these 12 patients. The authors make the interesting speculation that other mutations may account for type I disease, since only one of twelve children showed the SCL3A1 mutation.

In the Quebec population, type II patients (where heterozygotes excrete massive quantities of cystine) are uncommon. Because heterozygotes excrete more than 100 mmol cystine/g creatinine, type II patients may represent a heterozygous or type II/normal individual. Although these 2 children did not form stones in the first decade of life, they had family histories consistent with an autosomal

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dominant stone formation over 2 or 3 generations, respectively.

Many unanswered questions remain. There exists a need to clone and study the second cystine transporter present on chromosome 19 and determine its location in the nephron. Because of the degree of cystine excretion in heterozygotes, this transporter is presumably in the proximal tubule where bulk amino acid reabsorption occurs. The full story of SCL3A1 mutations remains to be uncovered. Do certain racial groups have unique mutations? Are there polymorphisms of this gene that anthropologists can employ to map historic migration patterns of man? Will "increase in function mutations" be found, such as in Liddle syndrome [10], that result in enhanced cystine reabsorption? Can we utilize the findings of this prospective study, where patients are identified by neonatal screening and genetic analysis, to better define preventative approaches to lessen or prevent stone formation. Studies emanating from the Quebec Screening Network have resulted in many achievements in the area of clinical investigations, especially for cystinuria [11]. These two papers represent a fulfillment of the promise.

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REFERENCES

1. Segal S, Thier SO: Cystinuria in *The Molecular and Metabolic Basis of Inherited Disease*, edited by Scriver CR, Beaudet A, Sly WS, Valle D, New York, McGraw-Hill, 1995, pp. 3581–3601
2. Scriver CR, Rosenberg LE: *Amino Acid Metabolism and its Disorders.* Philadelphia, W.B. Saunders, 1973 pp. 238–247
3. Goodyer P, Saadi I, Ong P, Elhas G, Rozen R: Cystinuria subtype and the risk of nephrolithiasis. *Kidney Int* 54:56–61, 1998
4. Saadi I, Chen XZ, Hediger M, Ong P, Pereira P, Goodyer P and Rozen R: Molecular genetics of cystinuria: Mutational analysis of SCL3A1 and evidence for another gene in the Type I (silent) phenotype. *Kidney Int* 54:48–55, 1998
5. Lee WS, Wells R, Sabag RV, Mohandas TK, Hediger MA: Cloning and chromosomal localizations of human kidney cDNA involved, dibasic and neutral amino acid transport. *J Clin Invest* 91:1959–1963, 1993
6. Pras E, Raben N, Golomb E, Arber N, Aksentijevich I, Schapiro JM, Harel D, Katz G, Liberman U, et al: Mutations in the SLC3A1 transporter gene in cystinuria. *Am J Hum Genet* 56:1297–1303, 1995
7. Zhang XX, Rozen R, Hediger MA, Goodyer P, Eydoux P: Assignment of the gene for cystinuria (SCL3A1) to human chromosome 2p21 by fluorescence in situ hybridization. *Genomics* 24:413–414, 1994
8. Wartenfeld R, Golomb E, Katz G, Bale SJ, Goldman B, Pras M, Kastner DL, Pras E: Molecular analysis of cystinuria in Libyan Jews: Exclusion of the SLC3A1 gene and mapping of a new locus on 19q. *Am J Hum Genet* 60:617–624, 1997
9. Kanai Y, Stelzner MG, Lee W-S, Wells, RG, Brown D, Hediger MA: Expression of mRNA (D@) encoding a protein involved in amino acid transport in S3 proximal tubule. *Am J Physiol* 263:F1087–F1093, 1992
10. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JR, Ulick S, Milora RV, Findling JW, Canessa CM, Rossier BC, Lifton RP: Liddle's syndrome: heritable human hypertension caused by mutations in the β subunit of the epithelial sodium channel. *Cell* 79:407–414, 1994
11. Goodyer PR, Clow C, Reade T, Girardin C: Prospective analysis and classification of patients with cystinuria identified in a newborn screening program. *J Pediatr* 122:568–572, 1993